Agrisera

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product AS01 017S

RbcL | Rubisco positive control/quantitation standard

product information

Background

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyzes the rate-limiting step of CO2 fixation in photosynthesis. It is one of the most abundant proteins on Earth and its homology has been demonstrated from purple bacteria to flowering plants.

Source of Rubisco standard: Rubisco protein was purified directly from a plant tissue - spinach.

Format

Lyophilized in glycerol

Quantity

100 µl

Reconstitution

For reconstitution add 95 µl of sterile water. Please notice that this product contains 10% glycerol and might appear as liquid but is provided lyophilized.

Storage

Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

Tested applications

Western blot (WB)

Related products

Primary antibodies matching Rubisco standard are:

AS03 037 | Anti-RbcL | Rubisco large subunit, form I and form II (50 µl). rabbit antibodies
AS03 037A | Anti-RbcL | Rubisco large subunit, form I and form II (50 µg affinity purified), rabbit antibodies

AS03 037-HRP| Anti-RbcL | Rubisco large subunit, form I and form II (40 µg, HRP-conjugated), rabbit antibodies

AS01 017 | Anti-RbcL | Rubisco large subunit, form I, chicken antibodies

AS15 2994 | Rubisco ELISA quantitation kit

collection of other protein standards

collection of other global antibodies
Plant and algal protein extraction buffer

Additional information

The RbcL protein standard can be used in a combination with Agrisera global antibiodies (AS01 017 from chicken or AS03 037 from a rabbit) to quantitate RbcL from a wide range of species. Global antibodies are raised against highly conserved amino acid sequence. This standard is also included in following kits: Educational antibody kit - photosynthesis, Photosynthesis Tool Kit - quantitation, Rubico quantitation kit,

Quantitative western blot: detailed method description, video tutorial

Application information

Recommended dilution

Standard curve: three protein standard loads are recommended.
For most applications a sample load of 0.2 µg of chlorophyll/well will give a RbcL signal in this range.

Positive control: a 2 µl load per well is optimal for most chemiluminescent detection systems. Higher standard concentration needs to be used to allow detection by Coomasie stains. Such gels with higher standard concentration can not be used for quantitation using chemiluminescence.

This standard **is stabilized** does not require heating before loading on the gel or addition of any buffer.

Please note that this product contains 10% glycerol and might appear as liquid but is provided lyophilized. Allow the product several minutes to solubilize after adding water. Mix thoroughly but gently Take extra care to mix thoroughly before each use, as the proteins tend to settle with the more dense layer after freezing.

Expected | apparent MW

52.7 kDa

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This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

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Additional information

Concentration: after re-constitution with sterile milliQ water final concentration of the standard is 0.15 pmoles/µl

Protein standard buffer composition: Glycerol 10%, Tris Base 141 mM, Tris HCl 106 mM, LDS 2%, EDTA 0.51 mM, SERVA® Blue G250 0.22 mM, Phenol Red 0.175 mM, pH 8.5, 0.1mg/ml PefaBloc protease inhibitor (Roche), 50 mM DTT.

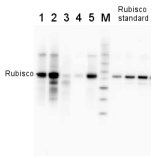
This standard is ready-to-load and does not require any additions or heating. It needs to be fully thawed and thoroughly mixed prior to using. Avoid vigorous vortexing, as buffers contain detergent. Following mixing, briefly pulse in a microcentrifuge to collect material from cap.
This standard is stabilized and ready and does not require heating before loading on the gel.
Please note that this product contains 10% glycerol and might appear as liquid but is provided lyophilized. Allow the product several minutes to solubilize after adding water. Mix thoroughly but gently Take extra care to mix thoroughly before each use, as the proteins tend to settle with the more dense layer after freezing.

Please, use the 55 kDa size of RbcL for calculations. The pmoles in the standard refer to pmoles of rbcL monomers.

Selected references

Poór et al. (2018). Comparison of changes in water status and photosynthetic parameters in wild type and abscisic acid-deficient sitiens mutant of tomato (Solanum lycopersicum cv. Rheinlands Ruhm) exposed to sublethal and lethal salt stress. J Plant Physiol. 2018 Dec 8;232:130-140. doi: 10.1016/j.jplph.2018.11.015.
Dai et al. (2018). Visualizing Individual RuBisCO and its Assembly into Carboxysomes in Marine Cyanobacteria by Cryo-Electron Tomography. J Mol Biol. 2018 Aug 20. pii: S0022-2836(18)30411-X. doi: 10.1016/j.jmb.2018.08.013.
Li et al. (2016). Interactive effects of nitrogen and light on growth rates and RUBISCO content of small and large centric diatoms. Photosynth Res. 2016 Aug 26. [Epub ahead of print]
Young et al. (2015). Antarctic phytoplankton down-regulate their carbon-concentrating mechanisms under high CO2 with no change in growth rates. Marine Ecology Progress Series 532:13-28.
Vandenhecke et al. (2015). Changes in the Rubisco to photosystem ratio dominates photoacclimation across phytoplankton taxa. Photosynth Res. 2015 Apr 11.
Wu et al. (2014). Large centric diatoms allocate more cellular nitrogen to photosynthesis to counter slower RUBISCO turnover rates. Front. Mar. Sci., 09 December 2014 | doi: 10.3389/fmars.2014.00068.
Li et al. (2014). The nitrogen costs of photosynthesis in a diatom under current and future pCO2. New Phytol. 2014 Sep 25. doi: 10.1111/nph.13037.

Application example



2 µg of total protein from various plant extracts (**1-5**) extracted with PEB (**AS08 300**) separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Markers MagicMarks (Invitrogen) (**M**) and Rubisco protein standard (AS01 017S) at **0.0625 pmol, 0.125 pmol, 0.25 pmol**.

Following standard western blot procedure this image has been obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad). The contour tool of the software is used to the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample.

Note: Optimal quantitation is achieved using moderate sample loads per gel lane, generally 0.5 to 2.5 ug total protein, depending on the abundance of the target protein.

Quantitation: When quantitated standards are included on the blot, the samples can be quantitated using the available software. Excellent quantitation can be obtained with images captured on the Bio-Rad Fluor-S-Max or equivalent instrument using Bio-Rad QuantityOne software. The contour tool is used to the area for quantitation and the values are background subtracted to give an adjusted volume in counts for each standard and sample. Using above protocol linear standard curves are generated over 1-1.5 orders of magnitude range in target load. It is important to note that immunodetections usually show a strongly sigmoidal signal to load response curve, with a region of trace detection of low loads, a pseudolinear range and a region of saturated response with high loads. For immunoquantitation it is critical that the target proteins in the samples and the standard curve fall within the pseudolinear range. Our total detection range using this protocol spans over 2 orders of magnitude, but the quantifiable range is narrower.
Quantitative western blot: detailed method description.